REPRODUCTION AND CYTOGENETICS IN ROMANOMERMIS CULICIVORAX (NEMATODA: MERMITIDAE)

BY

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Twelve chromosomes, two significantly larger than the rest, were observed in dividing hypodermal nuclei during the massive growth phase of female juveniles of R. culicivorax. The same number (2n = 12) was also detected in eggs deposited at the 5- to 64-cell stage of cleavage. Some hypodermal nuclei were polyploid (tetraploid or octaploid), evidently as a result of endomitosis. Maturation of oocytes was by regular meiosis leading to reduction of the chromosome number to n = 6. Re-establishment of the somatic chromosome number was by fusion of sperm and egg pronuclei. Non-inseminated females failed to produce mature eggs. It was concluded that reproduction was exclusively by cross-fertilization. However, the possibility that, under certain conditions, reproduction may be by gynogenesis could not be excluded entirely.

Keywords: Chromosomes, oogenesis, hypodermis, meiosis, genetics, insect parasites.

Sex determination in mermithids is density dependent, so that males predominate as parasite burdens increase. This acts to constrain growth of the parasite population and restricts their potential for biological control (Hominick & Tingley, 1984). Little information exists on the cytogenetic features accompanying this phenomenon, or the cytogenetic mechanism of sex determination. The present study of R. culicivorax was undertaken to discover its chromosomal complement and mode of reproduction in order to elucidate the chromosomal or genetic mechanism of sex determination, and so, how the environment can influence the direction of sexual differentiation of developing juveniles.

MATERIALS AND METHODS

Adult males and females, as well as third- and fourth-stage juveniles of R. culicivorax, were obtained from a culture maintained in Aedes aegypti larvae according to described procedures (Petersen & Willis, 1972). Two levels of infection were established; a high level under which a preponderance of males was obtained, and a low level yielding primarily females. The earliest harvest of sexually differentiated juveniles was made 70 to 100 hr post-infection, i.e. at

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a period of high division rate of hypodermal nuclei, and when male juveniles could be distinguished from female juveniles by their rudimentary spicule primordia. Juveniles from this rapid growth phase (see Curran & Webster, 1983) were designated 3rd stage, while postparasitic juveniles were designated 4th stage.

For processing, males, females and juveniles were smeared on glass microscope slides before being submerged in 1N hydrochloric acid for 2 min. The slides were then transferred to a 3:1 absolute alcohol-acetic acid mixture for 15 to 20 min. Excess fixative was drained from the slides and a drop of 2% propionic orcein was placed on the nematode material (Triantaphyllou, 1979). To prevent evaporation during the 30 minute period of staining, a depression slide was inverted over the material. Excess stain was then drained off the slide and a coverslip was applied. The mount was sealed with a 1:1 paraffin-lanolin mixture. Valuable mounts were made permanent according to the rapid freeze method (Triantaphyllou, 1985).

Advanced stages of maturation of oocytes were studied in eggs deposited overnight, or in mature eggs dissected out of the uteri of females. Such eggs had developed a thick impermeable eggshell and required treatment to increase their permeability to the fixative and stain. They were exposed for 4-6 min to a mixture of 0.1N KOH in 0.4% NaOCl and then washed thoroughly in three changes of distilled water. Finally, the eggs were concentrated in a few drops of 1% gelatin solution. A drop of the egg suspension was transferred to the centre of a microscope slide, spread onto a larger area (about 1 cm square) and allowed to dry for 10-15 min. Slides were then processed for staining, starting with hydrolysis and fixation, as previously described (Triantaphyllou, 1985).

RESULTS

Somatic Chromosomes. — The somatic chromosome number of 2n = 12 was observed in dividing hypodermal nuclei of male and female third-stage juveniles dissected from mosquito larvae and in eggs deposited at the 5- to 64-cell stage of cleavage. Metaphase figures of dividing hypodermal nuclei were the most favourable for revealing the karyotype of the juveniles. The diploid chromosomal complement comprises two long chromosomes and 10 much shorter ones (Fig. 1A, B). The short chromosomes are rod-shaped, indistinguishable from each other and lack distinct morphological details, such as an organised centromere. The long chromosomes are the last to become condensed at late prophase (Fig. 1A). They are among the first to divide during early anaphase, when they show the two chromatids lying parallel to each other. This behaviour often gives them the appearance of simple metacentric chromosomes (Fig. 1B). Many divisions of hypodermal nuclei were incomplete (endomitotic), resulting in nuclei of higher degree of ploidy